Instrument Manual

LKB

LKB 2138 Uvicord® S

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PLEASE READ THIS MANUAL CAREFULLY BEFORE ATTEMPTING TO USE THE INSTRUMENT.

In addition to this Instrument Manual you are supplied with a Spare Parts Catalogue. Upon request, to the address below, a Service Information Manual will be made available.

However, LKB can only be responsible for the effects, on safety and performance, of modifications or repairs carried out by us or in accordance with our approved specifications.

If you have any comments on this manual, we will be pleased to receive them at:

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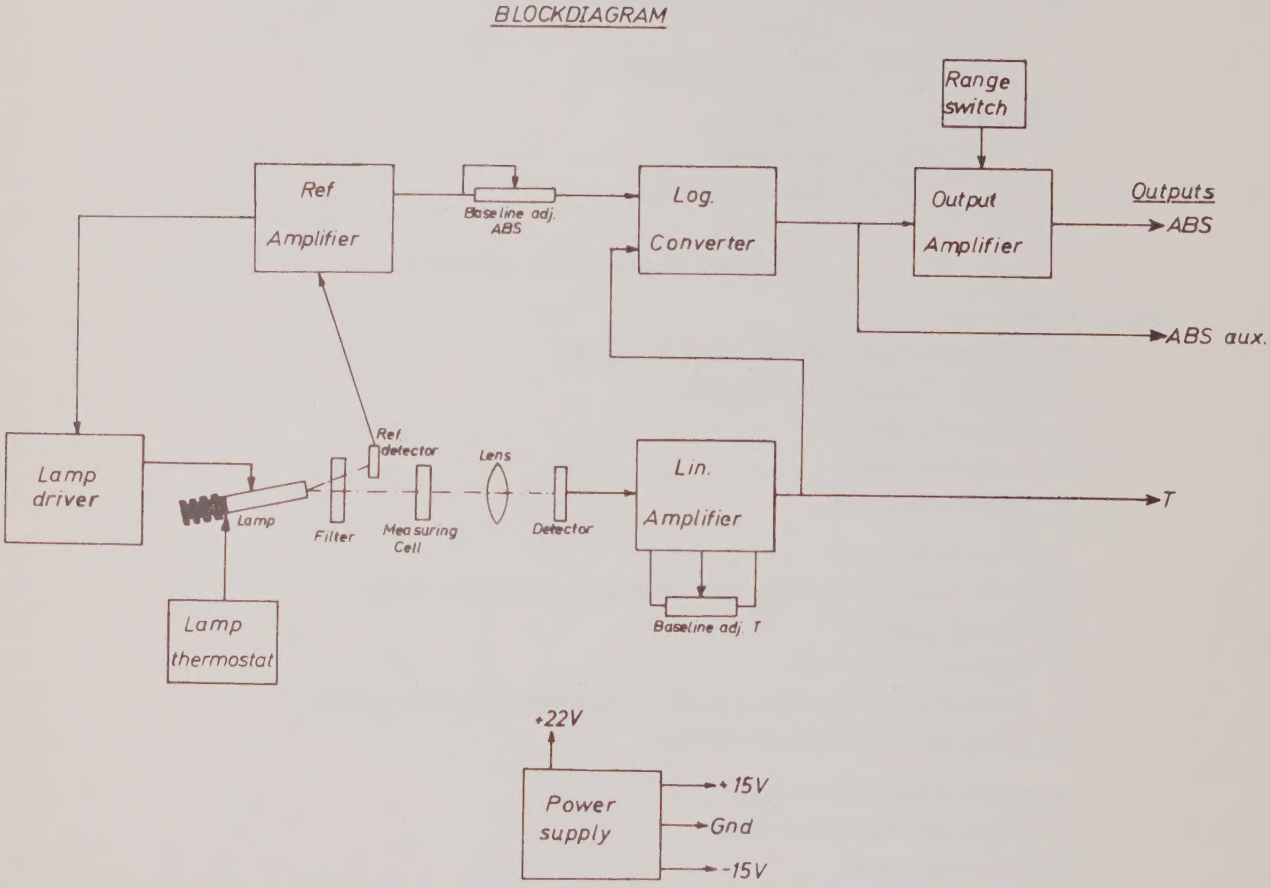

BLOCKDIAGRAM
Range switch
Ref Amplifier
Baseline adj. ABS
Log. Converter
Output Amplifier
Outputs
ABS
ABS aux.
Lamp driver
Ref detector
Lens
Lin. Amplifier
T
Lamp
Filter
Measuring Cell
Detector
Baseline adj T
Lamp thermostat
+22V
Power supply
+15V
Gnd
-15V

Fig. 1

I. INTRODUCTION

LKB 2138 Uvicord S is a single-beam UV monitor with flow-through cell for monitoring the eluants from various separation columns. Available wavelengths, selected by combination of lamp and interference filter, are 206, 254 and 280 nm.

LKB 2138 Uvicord S is built up in one combined optical/electronic unit, and outputs simultaneously both transmission values (0—100%) and absorption values (0.005-2A full scale deflection), selectable in 9 ranges. Thus, by selection of the appropriate absorbance range, Uvicord S makes it possible to monitor both high and low concentration substances in the same sample.

Operating at 206 nm, it is possible to monitor many substances not covered by conventional UV monitors. Substances such as non-aromatic peptides, amino acids, lipids, nucleotides and certain carbohydrates have strong absorption at 206 nm, whereas at higher wavelengths they show little or no absorption. Furthermore, most proteins also exhibit greater absorption at 206 nm than they do at 280 nm.

By employing all solid state electronics and the very latest detection principles, Uvicord S performs to specification at ambient temperature and under cold room conditions.

II. PHYSICAL DESCRIPTION

Basic Principle (See Block Diagram, Fig. 1)

The light from the high frequency excited gas discharge lamp is filtered by the interference filter before it passes the measuring cell. The light transmitted through the measuring cell is then focused on the measuring detector and converted to an electrical signal directly proportional to the light intensity. This signal is amplified in the following linear amplifier, the gain of which is controlled by the Baseline Adjust "T" knob. The gain is set to give 100 mV output with only buffer in the measuring cell. This signal is the transmission (%T) output.

The filtered light also falls on the Reference Detector, the signal of which is amplified in the reference amplifier. As the reference signal is proportional to the light intensity, it is used to control the lamp driver for obtaining constant and stable light.

The reference signal is also connected to the Logarithmic Amplifier via the Baseline Adjust "ABS" potentiometer and used as reference for the lin-log conversion (I_0).

The Log. Converter receives on its two inputs, the reference signal and the measured signal. The ratio between the two signals is transformed by the converter, and connected directly to both the output amplifier and the aux. output socket. The gain of the output amplifier is controlled by the range switch in order to obtain output ranges 0.005-2A. The output amplifier is offset to give 100 mV output, corresponding to OA.

In order to ensure stable emission and long lamp life, the lamp holder is thermostatted. The thermostat is set to switch on heating when the lamp temperature goes below room temperature (approx. 22° C).

The electronics are powered by +15V and −15V.

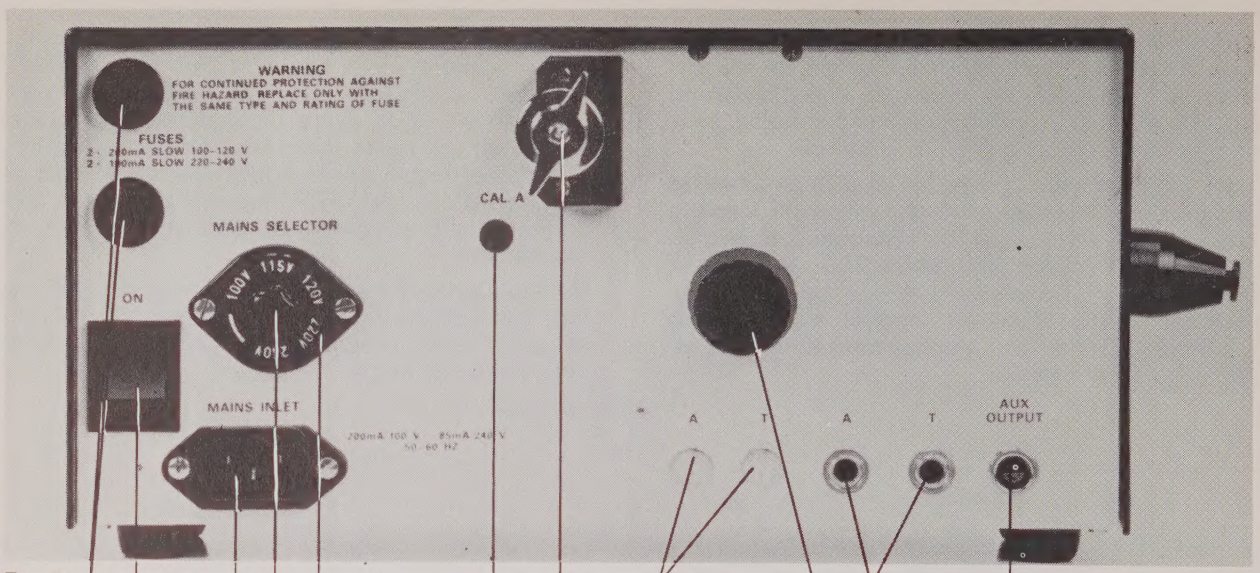


Fig. 2

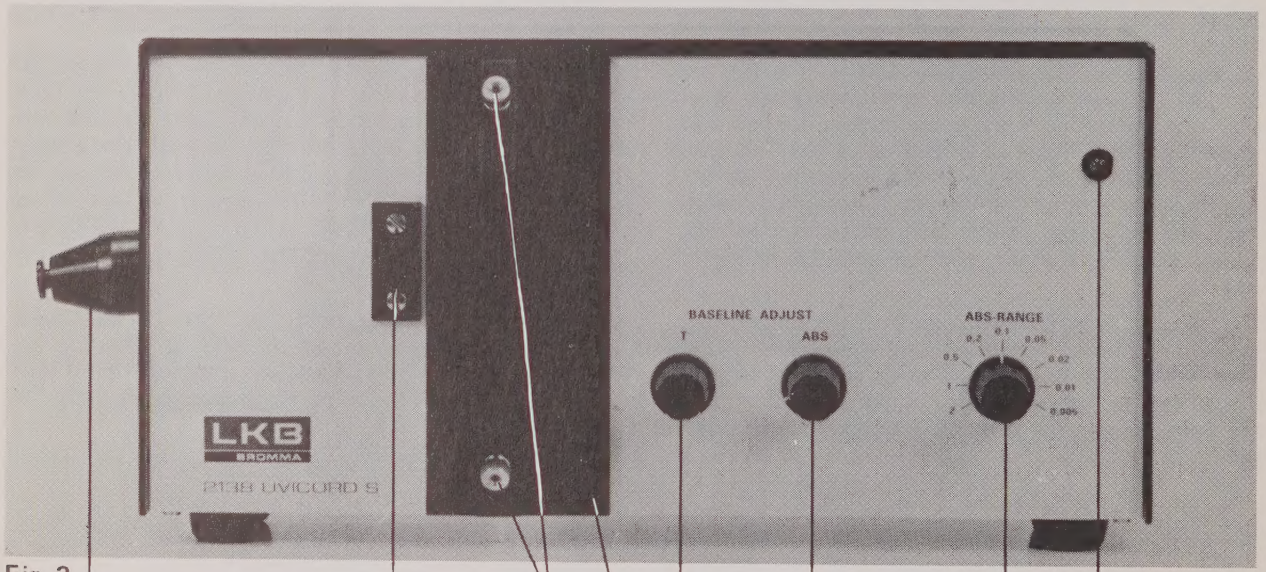


Fig. 3

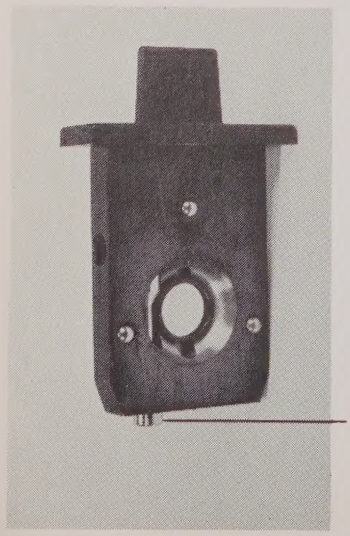


Fig. 4

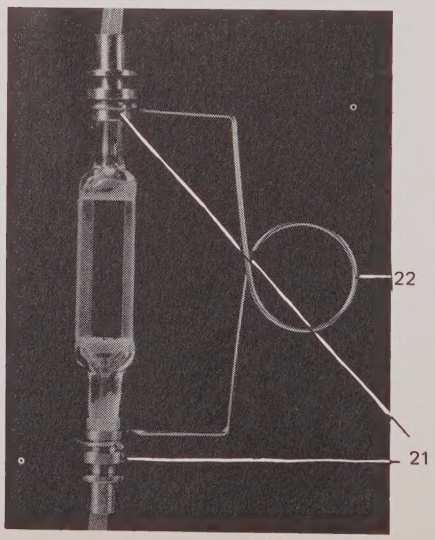


Fig. 5

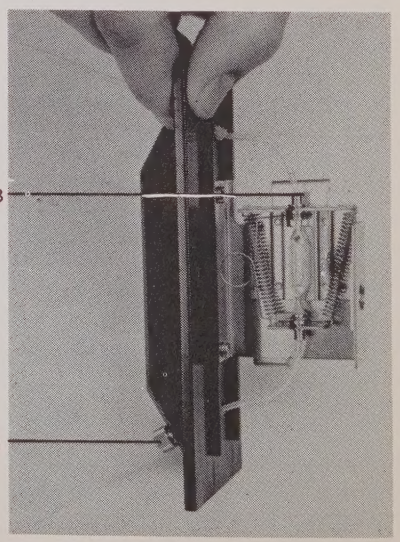


Fig. 6

Front panel (Fig. 2)

(12) Lamp Holder— The chosen monitoring wavelength determines which lamp shall be used. Each lamp type is identified by a figure (1) or (2) on the end of the lamp.
Lamp 1 for wavelengths 206 and 280 nm.
Lamp 2 for wavelength 254 nm.

(11) Filter Holder— The filter holder has a spring-loaded locking mechanism for quick release of the filter. The holder can be inserted into the instrument either way.

(17) Cell Holder— The complete cell holder is quickly removed by unscrewing knob (6) on the rear panel. The standard flow cell has optical pathlengths of 2.5 mm or 5.0 mm. Tubing access to the flow cell is through the two ports (18).

Control Knobs (16) — adjusts transmission baseline.
(15) — adjusts absorbance baseline.
(14) — selects desired absorbance range.

(13) Indicator Lamp— Indicates mains voltage is ON.

Rear panel (Fig. 3)

All electrical connections and switches are located on the rear panel

(0)— mains input.

(9)— mains ON/OFF switch..

(1)— mains voltage selector with circuit breaker (2).

(3)— fuse holders — ensure that fuses of the correct rating are inserted.

(7)— calibration potentiometer.

(8)— mounting bracket.

(6)— cell holder release and optical alignment control (see point 18, page 11).

(4)— absorbance and transmission outputs for potentiometric recorders (i.e. LKB 2210 Recorder).

(5)— absorbance and transmission outputs for galvanometric recorders (i.e. LKB 6520 Recorder).

(10)— auxiliary output providing — 1 volt per absorbance unit.

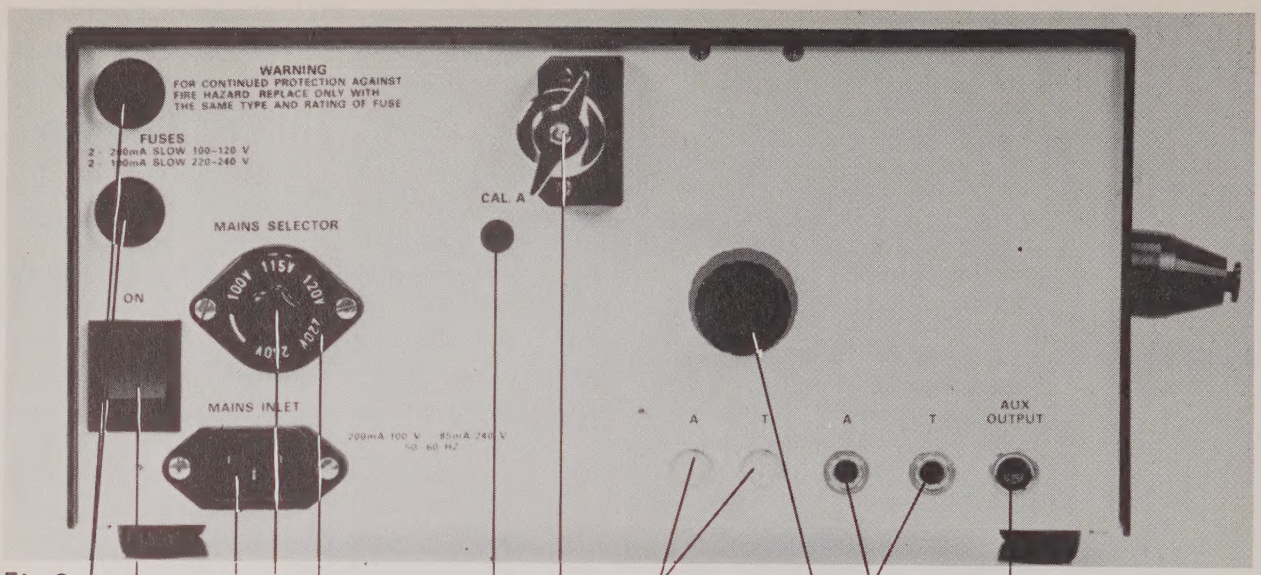


Fig. 2

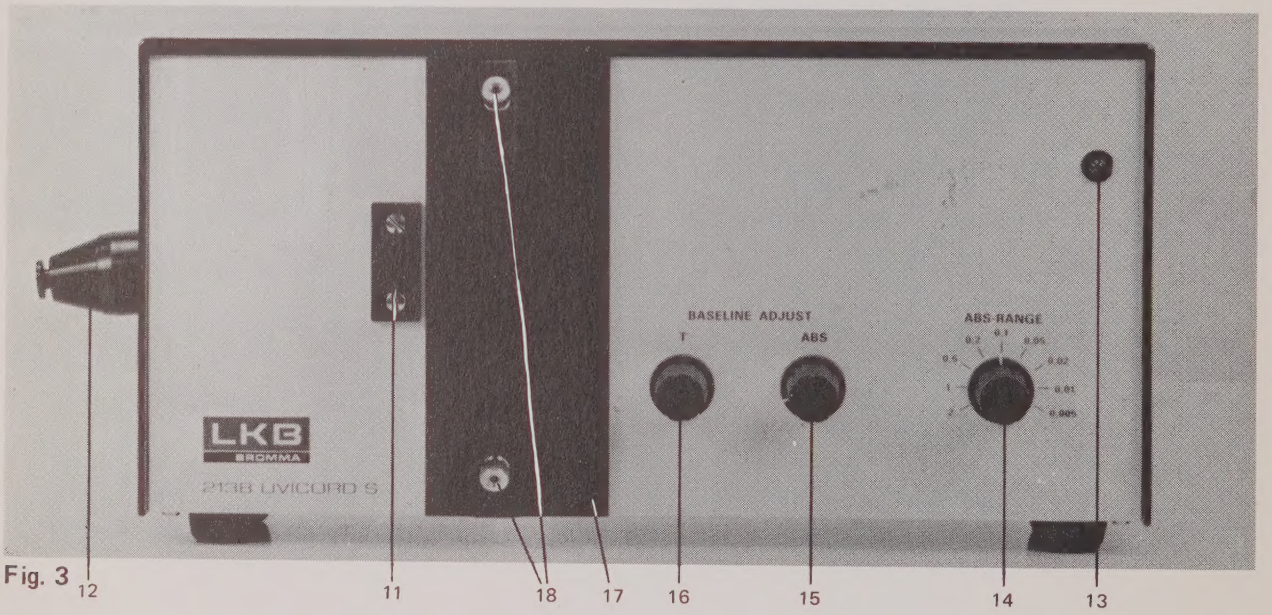


Fig. 3

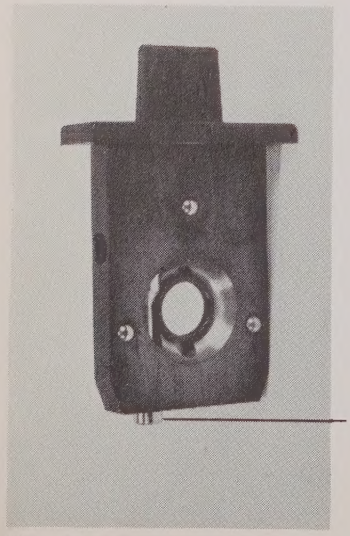


Fig. 4

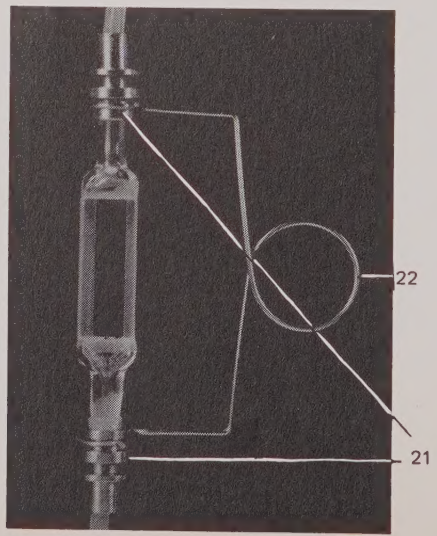


Fig. 5

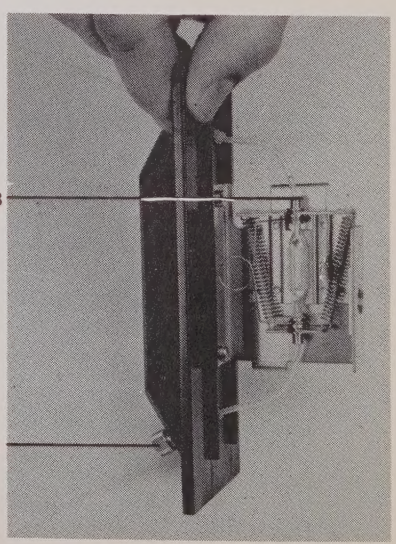


Fig. 6

III. INSTALLATION & MAINTENANCE

General precautions

Environmental conditions

The UVICORD S is designed for use in normal laboratory conditions and should not be installed where the atmosphere is unduly moist, corrosive or contaminated by vapours which may form deposits on components in the optical path.

To minimize drift, the temperature should be kept constant. Thus, the UVICORD S should not be placed in a draught, in direct sunlight or in the vicinity of warm apparatus or fans, where temperature fluctuations may occur.

Pay particular attention not to place the instrument near the compressor fan or in the fan stream in cold boxes or coldrooms.

Lamps

Once installed and stabilized, the UVICORD S should not be bumped or tilted and the lamp should not be touched, as this may cause a disturbance in the distribution of material in the lamp, resulting in an undesired shift of light intensity.

WARNING: If a Lamp 2 should become broken, make sure that all Hg and glass are removed to avoid mercury poisoning.

Filters & Flow Cells

Special care must be taken when handling interference filters. They should not be exposed to temperatures exceeding 60° C, nor should they be allowed to come in contact with any kind of liquid.

Clean the filters only with lens cleaning tissue. Other optical surfaces may be cleaned by wiping the surface with a clean, lint-free cloth moistened with carbon tetrachloride, ethanol, or some other suitable pure solvent.

For cleaning of interior of flow cells see "Cleaning of flow cells".

Before Use

When unpacking the instrument, please check with the packing list to ensure that all items are included and in good condition.

Setting-up

1. Check that the rear voltage selector (1) is set to the correct voltage. If it is not, unscrew the center circuit breaker (2) and turn the red mark to the correct voltage with the help of a coin or screw-driver and reinsert the center circuit breaker (2).
2. Insert the appropriate fuses into the fuse holders:

   200 mA SLOW for 100-120 V, or

   100 mA SLOW for 220-240 V.
3. Connect the recorder cable(s) to the desired output connector(s) on the rear.

   Outputs (4) are for 100 mV-recorders and

   Outputs (5) are for 0.3 mA-recorders (LKB 6520-series).

   Note 1: OUTPUTS 4 & 5 *must not* be used simultaneously.

   Note 2: For 6520-recorders, the signal cable must be reversed i.e. red plug goes into black connector on the recorder.
4. Mount the desired filter into the filter holder (11) (Fig. 2). Depress the lever (24) and insert the filter gently with the help of forceps. Release the lever (24) to hold the filter firmly in position.

   Always make sure that both sides of the filter are clean (use lens tissue paper to clean) and never touch the surfaces with your fingers.

   Note 1: It does not matter which side of the filter faces the lamp.

   Note 2: It does not matter which way the filter holder goes in.
5. Insert the appropriate lamp (Lamp 1 for 206 or 280 nm: Lamp 2 for 254 nm) into the lamp holder (12).

   Note 1: The lamp should be fully inserted into the holder.

   Note 2: Lamp 2 is only supplied as standard with LKB 2138-003 and 2138-004 for HPLC.
6. Remove the cell holder (17) by turning the knob (6), on the rear, counter-clockwise.

Fig. 7

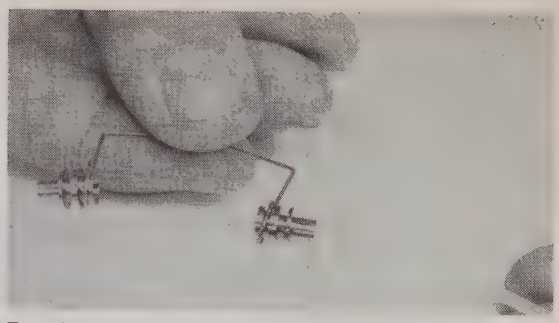


Fig. 8

Fig. 9

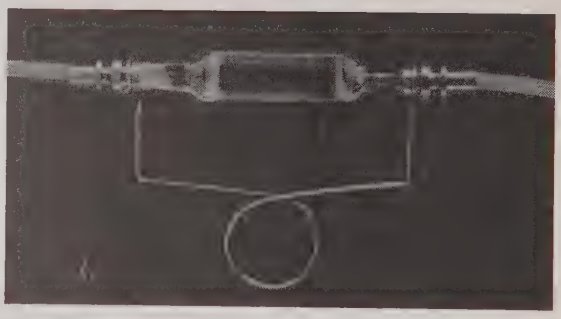


Fig. 10

Fig. 11

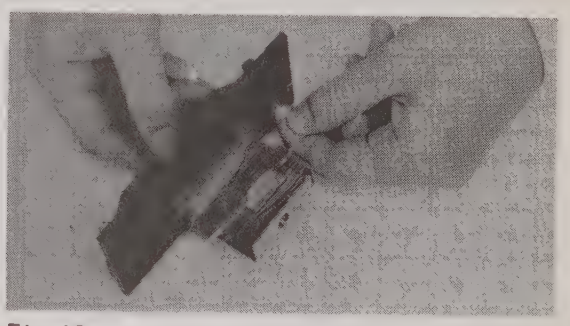


Fig. 12

Fig. 13

Fig. 14

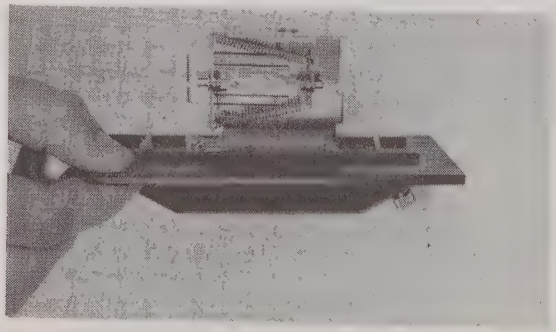


Fig. 15

7. Snap the cell collars (21) into the cellholder spring (22) (Fig. 7).
8. Thread the two pieces of flanged tubing through the cell collars (22) (Fig. 8).
9. Insert the flow cell between the cell collars (21) (Fig. 9 and 10).

   Note: The flow cell should be cleaned before insertion (see "Cleaning of flow cells").
10. Clean the cell with lens cleaning paper.
11. Insert the cell holder spring (Fig. 10) into the spring-loaded holder by depressing the lever (23).
12. Turn the cell so that it *lies flat against the aperture hole*. Do not touch the cell with your fingers.
13. Insert the tubing through the inlet and outlet holes of the cell holder (Fig. 12).

    Note: It is usually necessary to unscrew the fastening screws (18) and to remove the small slotted plastic cone in order to be able to thread the tubing through.

    Then thread the tubing through the cone and the fastening screws (18) (Fig. 13).
14. Fix the tubing by tightening the fastening screws.
15. Reinsert the flow cell holder (17) (Fig. 15) into the instrument, by tightening the knob (6) on the rear panel.
16. Connect the instrument to mains voltage and switch it on with mains switch (9).
17. Check that the lamp lights up by looking directly into the lamp from behind, through the "handle".

    Note: If the lamp does not ignite, pull out the lamp, and rub the glass portion gently against a cloth, in order to produce static electricity. Re-insert the lamp.

    Note: Always make sure that there is a filter in the filter holder, before trying to ignite the lamp.
18. Since it is knob (6) which is positioning the flow cell in the optical path, knob (6) should be adjusted to produce maximum transmission deflection on the recorder.

Stabilization time

The lamps normally require a stabilisation time of 30 min for work in the absorbance ranges 2A to 0.5A, and 4 hours for work in the ranges 0.2A to 0.005A. It is thus recommended to leave the instrument switched on continuously, unless longer (i.e. weeks) periods of non-use are expected.

Important Note: When operating in cold rooms, i.e. at temperatures below 10° C, make sure that the instrument is placed in the cold room at the desired running temperature 12 hours before a run. This is necessary to lower the temperature of the instrument housing. Once this has been done the stabilization times stated above are valid, i.e. if an instrument is left in a cold room it will have a short stabilization time when it is switched on.

Recorders

The baseline (0 Absorbance units or 100 % Transmission) is located at the right-hand side of the recorder, so that a time-axis running from left to right is obtained.

Adjustment of zero setting (baseline)

Note: The transmission adjustment MUST ALWAYS be performed. If a one-channel recorder is used, first connect it to the %T output and adjust the baseline according to the following, and then connect it to the absorbance output and adjust the absorbance baseline.

Note: Zero adjustment should be made with the buffer to be used flowing through the flow cell.

For LKB 2210 or 6520-recorders (normal paper)

1. Adjust the zero position (left side of the chart) on the recorder by short-circuiting the input(s) or depressing the zero check button on the recorder. Select the 100 mV range on LKB 2210 recorder.
2. Adjust the transmission baseline (right side of the chart) with the T-knob (16), until a deflection of 100% (=100 mV) is achieved. The instrument is now ready for use with the % T-output.
3. Turn the absorbance range selector (14) to position 0.01A or the desired range.
4. Adjust the absorbance baseline (right side of the chart) with the A-knob (15), until a deflection of 100 mV is achieved.
5. Because of the tolerance in calibration of the recorder and the 100 mV-output, check that the baseline is correct by switching the absorbance range selector from 0.01A to 2A. The baseline should remain stable during this procedure. If not, switch back to the 0.01A-range and turn the A-adjustment knob in the opposite direction to the deflection. Repeat this procedure until the baseline remains stable. This may be slightly away from the 100 mV-line on the chart paper.
6. Select the desired absorbance range and start the run.
7. The baseline can now be displaced by turning the zero knob on the recorder.

For LKB 2210 (with special chart paper 2210-120, with offset baseline)

1. Adjust the zero position on the recorder by short-circuiting the input(s) or depressing the zero check button on the recorder. Select the 100 mV-range on the LKB 2210 recorder.
2. Adjust the transmission baseline with the T-knob (16) until a recorder deflection of 100 mV (=right hand edge of paper scale) is achieved.
3. Turn the absorbance range selector (14) to position 0.01A.
4. Adjust the absorbance baseline with the A-knob (15), until a deflection of 100 mV is achieved (=right hand edge of the paper).
5. Because of the tolerances in calibration of the recorder and the 100 mV-output, check that the baseline is correct by switching the absorbance range selector from 0.01A to 2A. The baseline should remain stable. This may be slightly away from the right hand edge of the paper.
6. Depress the 200 mV-button of the recorder.
7. Depress the VAR-button of the recorder.
8. Adjust the VAR-knob of the recorder until the baseline is on the 0.0 (=100) line.
9. Select the desired absorbance range.

NOTE: 100 mV = 100% T
or
0,0 A
0 mV = 0% T
A as selected

The UV-monitor in a chromatography system

A UV-monitor is the simplest and yet the most universal of all chromatography detectors. It can be employed as an analytical instrument with high sensitivity and wide-ranging detectability as well as a rather crude monitor for preparative work.

In any chromatographic set-up, the UV-monitor must be considered as an integral part, and the chromatographic conditions must be chosen to fit not only the actual separation but also the parameters of the detector. The parameters which must be considered are:

Selection of operating wavelength

When choosing the operating wavelength, one must consider both sample and buffer spectra and concentrations. Generally speaking, operation at short wavelengths increases detectability and sensitivity, i.e. more peaks are seen and smaller amounts are detected.

1. *Unknown samples* are best detected at 206 nm, since virtually all biochemical compounds have some UV-absorbance at 206 nm and one is therefore assured of not missing any peaks because a compound does not absorb at, for instance, 254 or 280 nm.
   Furthermore, detection at 206 nm yields much higher sensitivity than 254 or 280 nm since the extinction coefficients for biochemical compounds, at 206 nm, are usually 2 to 200 times greater than those at 254 or 280 nm. This means that trace components which are completely undetected at 254 and 280 can be detected at very small concentrations at 206 nm.
2. *Known samples* are best detected at the wavelength where all the peaks of interest to the chromatographer can be monitored at a satisfactory sample concentration. Whereas any compound is best detected (i.e. lowest concentration necessary) at its extinction maximum, the problem for the chromatographer is to find a wavelength which gives a strong enough extinction by *all* the compounds which are to be detected. In this respect, 206 nm offers strong advantages over both 254 nm and 280 nm, particularly as the sample concentrations, when monitored at 206 nm, need only be 1/10th, or less, of the concentrations required to give the same peak height at 254 or 280 nm.
3. *Buffer conditions*
   Buffers, as well as most other compounds, absorb more strongly at 206 nm than at 254 nm or 280 nm. Thus the choice of buffer system is a deciding factor, governing the degrees of detectability and sensitivity achievable.
   The light source intensity of UVICORD S is so high that background absorption (due to buffers) can be "backed off" up to concentrations corresponding to 0.4 A at 206 and 254 nm with a 2.5 mm cell. Thus the UVICORD S can be used with quite strongly absorbing buffers.
   Should the buffer absorption be too strong in a 5 mm cell, a reduction of pathlength down to, for instance, 0.5 mm will give a 10-fold increase in buffer concentration that can be backed off. The amplification obtained by changing from absorbance range 2A to absorbance range 0.005A would, in this case, still ensure that sensitivity is not lost.

Selection of flow cell

1. *Cell volume and influence of volume of connecting tubing*
   For both preparative and analytical chromatography it is important to minimize peak broadening due to large mixing volumes in the flow cell and connecting tubing. One should therefore, as a general principle, choose the shortest possible length of connecting tubing with the smallest possible inner diameter when setting up the detector. Furthermore, the flow cell should have as small a volume as possible; however, bear in mind here the restrictions on cell volume already set by choice of optical pathlength due to buffer background absorption or sensitivity requirements. A variety of flow cells can be obtained (see Appendix C).
2. *Optical pathlength*
   A range of cells, with optical pathlengths from 0.5 to 5.0 mm, is available. Selection of appropriate optical pathlength is a means of optimizing sensitivity in accordance with buffer background absorption, sample concentration and the linear dynamic range of the detector.

   For applications with a strongly absorbing buffer, one would choose a short pathlength, e.g. 0.5 mm, and compensate for the ensuing sensitivity loss by employing an amplified absorbance range such as 0.01 or 0.005A.

   In cases where the sample concentration can be freely chosen, one should strive to work with as long a pathlength as possible, i.e. 5 mm, in order to minimize noise. Furthermore, one should try to work with an absorbance range which is optimal from a linearity point of view, i.e. 0.005 to 0.5A.

   In cases where the sample concentration is low, one should strive for long pathlengths and employ amplified absorbance ranges. For a quantitative discussion on detection limits, see "Selection of Absorbance Range".

In cases where the sample concentrations vary greatly and it is desirable to detect both strongly and weakly absorbing components, one should employ the longest possible path-length and the highest possible amplification. In combination with this, one should also employ the dual outputs of %T and the selected absorbance range, so that both large and small peaks can be recorded simultaneously.
See "Signal Outputs".

Cleaning of flow cells
Clean flow cells are essential to the proper operation of the Uvicord S. Cells should not be allowed to dry out if they contain liquids with dissolved salts, proteins or other components with low volatility. Particles should not be allowed to dry out if they contain liquids may be inspected for particles by removing the cell holder and examining the cell with a magnifying glass.

If the cell contains *trapped particles,* proceed as follows:

1. Remove the cell holder from the Uvicord S.
2. Connect a syringe to the outlet tubing and flush the cell with small volumes of 50 % (v/v) ethanol in water. Examine the cell from time to time, to check that all the particles have been washed out.
3. Rinse the cell with about 100 ml of distilled water, and replace it in the Uvicord S.
4. Reconnect to the chromatography system.

Most *non-particulate contaminants,* e.g. denatured proteins, salts, etc. can be removed by flushing the cell with a warm laboratory detergent solution or other appropriate solvent; whichever is used, the cell should then be thoroughly rinsed with distilled water or clean solvent.

Oily deposits, which increase the tendency to trap bubbles, can be removed by rinsing the flow cell first with a non-polar solvent (e.g. hexane), then with a polar solvent (e.g. isopropanol), and finally with distilled water. If the flow cell has been used with aqueous solutions, it should be rinsed in the following sequence: warm aqueous laboratory detergent solution, distilled water, polar solvent, non-polar solvent, polar solvent, distilled water.

Persistant contaminants may be removed with cleaning acid (chromic acid), as follows:

1. Prepare fresh chromic acid by adding 100 ml concentrated sulphuric acid to 3.5 ml of a saturated solution of sodium dichromate.
   Warning: Chromic acid is extremely corrosive. Dilute spilt acid immediately with a large excess of water.
2. Remove the cell holder from Uvicord S.
3. Connect a glass syringe to the outlet side of the cell, and carefully draw chromic acid into the cell. Do not draw acid into the syringe.
4. Leave the acid in the cell for 10–20 minutes. (Exposure to the acid for several hours, if absolutely necessary, will not harm the cell).
5. Carefully remove the cleaning acid, and rinse the cell in the following sequence: 100 ml distilled water, 100 ml 50 % (v/v) ethanol in distilled water, 100 ml distilled water.
6. Replace the cell holder in the Uvicord S, and reconnect to the chromatography system.

4. *Prevention of microbial growth in the chromatography system*
The risk of microbial growth is slight when the system is in use, but high when the system is standing idle. Antimicrobial agents may be used as a pre-run rinse. The agent chosen should not interact either with the sample or with the gel. Whichever antimicrobial is used, at least one column volume of buffer should pass through the monitor flow-cell before the baseline is adjusted.

Sodium azide (NaN_3), 0.02 %, does not interact noticeably with proteins or carbohydrates, and does not change their chromatographic behaviour. It interferes with fluorescent marking of proteins and the anthrone reaction, and inhibits certain enzymes.

Chloretone (trichlorobutanol), 0.05 %, is only effective in weakly acidic solutions.

Merthiolate (Thimerosal, ethylmercuric thiosalicylate), 0.005 %, is most effective in weakly acidic solutions. It binds to, and can inactivate, compounds containing thiol groups.

Hibitane (Chlorhexidine), 0.002 %, is very effective and is incompatible with only a few substances.

Phenylmercuric salts (acetate, borate, nitrate) 0.001 to 0.01 %, are effective only in weakly alkaline solutions.

Chloroform, butanol and toluene are not recommended if the chromatography system contains plastic materials, or when using certain dextran gels. They are effective only in very concentrated solutions.

5. *Prevention of bubble formation*
The design of the LKB flow cells eliminates the problem of bubbles being trapped in the optical path.

In order to avoid bubble formation totally, one should:

a) use only properly degassed buffers.

b) try to increase the pressure at the outlet of the flow cell, for example by using a length of narrow bore tubing.

c) always let the direction of flow through the flow cell be from the bottom upwards (with the exception of density gradient runs).

d) let the buffer temperature be slightly higher (3–5° C) than the column temperature, which means that the buffer will be cooled in the column, thus increasing its capacity to absorb gases.

If a bubble, in spite of these measures, should form, it is normally flushed out immediately

and will only cause a spike on the recorder chart.

If the above measures are not taken and a bubble forms and stays in the flow cell, it is easily removed by applying pressure to the outlet tubing.

Selection of Absorbance Range

1. *Choice of absorbance range*
In most cases it is very difficult to determine the best absorbance range a priori.
However, knowing an approximate concentration level one can use the Lambert-Beer's law:

$A = \epsilon_1^{\lambda} \cdot l \cdot c$

where

ϵ_1^{λ} = molar extinction coefficient at wavelength λ, measured with a 1 cm optical pathlength (l/mole. cm).

l = optical pathlength in cm.

c = concentrations of sample in moles/liter.

Knowing the approximate concentration level and from a UV-atlas the ϵ-value, one can determine the approximate absorbance level. One must, however, also estimate the dilution factor generated by the chromatography system (usually 5 to 100 times).

An example:

The ϵ_1^{254}-value for $K_2Cr_2O_7$ taken from a UV-atlas is 4310.
The column dilution factor is estimated to be 10.
The optical pathlength of the flow cell is 5 mm (0.5 cm).
The sample concentration is estimated to be 0.1 mM.

Thus,
$A = 4310 \times 0.5 \times 0.1 \times 10^{-3}$ absorbance units
$\approx$ 0.2155 absorbance units

With a dilution factor of 10 one would then estimate the absorbance to be roughly 0.02A, i.e. the absorbance range 0.05A should be used.

2. *Detection Limits*
Conversely to the above discussion on absorbance ranges, one can estimate what the concentration limit is for a substance, once the following are known:

a) the noise level peak-to-peak with flowing buffer,
b) the ϵ_1^{λ}-value,
c) the optical pathlength and
d) the dilution factor.

The detection limit is defined as the concentration which produces a peak which is distinguishable from baseline noise. To be distinguishable, it is necessary that the signal level be twice the baseline noise measured peak-to-peak.

i.e. rewriting Lambert-Beer's law $c = \frac{A}{l \cdot \epsilon_1^{\lambda}}$

Concentration detection limit

$$= \frac{2 \times \text{noise peak-to-peak}}{l \cdot \epsilon_1^{\lambda}}$$

Example
With a noise level (with flowing buffer) peak-to-peak of 0.0001A and $\epsilon_1^{254} = 4310$ ($K_2Cr_2O_7$), dilution factor 10, optical pathlength 0.5 cm, the concentration detection limit

$$= \frac{2 \times 0.0001}{0.5 \times 4310} \times \frac{\text{moles}}{1} \approx 90 \text{ nanomoles/liter}$$

With a dilution factor of 10, ten times as high a concentration is required for sample application i.e. 0.9 μ moles/liter

Note that, using 206 nm, where ϵ_1^{206} for $K_2Cr_2O_7$ = 10200, would have yielded a concentration detection limit of 0.4 μ moles/liter of $K_2Cr_2O_7$
i.e. at 206 nm the detector is more sensitive for $K_2Cr_2O_7$ than at 254 nm.

3. *Calibration*
In order to check that the filter has not been degraded (i.e. that its transmission level has not fallen) and to check that there is no absorbing deposit in the optical path, the instrument can be calibrated against a known standard. Calibration is also necessary when comparisons between chromatograms run with different pathlengths are required. In this case, one can adjust the calibration to compensate for tolerances in the nominal pathlengths of the flow cell.

The calibration substance recommended is $K_2Cr_2O_7$, dissolved in 0.05N H_2SO_4 which has a spectrum suitable for all three wavelengths: 206, 254 and 280 nm.

$K_2Cr_2O_7$ gives the following ϵ_1^{λ} -values:

$\epsilon_1^{206} = 10200$

$\epsilon_1^{254} = 4310$

$\epsilon_1^{280} = 3300$

Thus with a 2.5 mm optical pathlength cell, the following concentrations should give a 0.4A deflection:

0.157 mM $K_2Cr_2O_7$ in 0.05N H_2SO_4 at 206 nm
0.371 mM $K_2Cr_2O_7$ in 0.05N H_2SO_4 at 254 nm
0.485 mM $K_2Cr_2O_7$ in 0.05N H_2SO_4 at 278 nm

Calibration procedure 1: Calibrates %T against absorbance.

1. Let the instrument run with dry cell or with distilled water in the cell.
2. Zero the instrument as described in "Adjustment of zero setting".
3. Select the 1A absorbance range.
4. Unscrew the flow cell holder knob (6) until a 10 % transmission level is reached.
5. The absorbance signal should now show a deflection of exactly 1.0A.
If not: Adjust the recorder deflection to

1.0A by turning the calibration screw (7) (situated behind the rubber plug) on the rear panel.

6. Tighten the flow cell holder again and zero the instrument again (see "Adjustment of zero setting").

The transmission and absorbance scales are now calibrated, i.e. their values are interchangeable and can be converted via logarithmic conversion.

Calibration procedure 2: Calibrates absorbance output against known standard.

1. Zero the instrument as described in "Adjustment of zero setting".
2. Select the 0.5A absorbance range.
3. Fill the flow cell with the appropriate concentration of $K_2Cr_2O_7$, see previous page.
4. Check that the absorbance is 0.4A.
   If not: Adjust the recorder deflection by turning the calibration screw (7) (situated behind the rubber plug) on the rear, until it shows 0.4A.

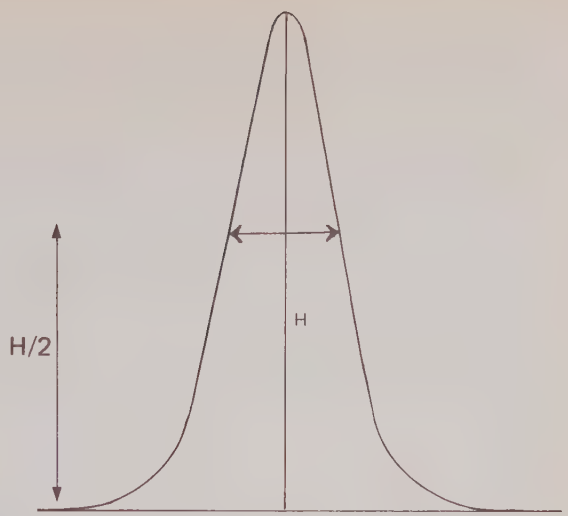
   If it is impossible to reach a 0.4A level, check that the optical pathway is clear.

The absorbance scale is now altered to compensate for tolerances in the optical pathlength. This may alter the calibration of %T and absorbance and it is recommended to use any of the alternative outputs for getting high and low sensitivity. See "Signal Outputs".

4. *Comparison of chromatography peaks*

In order to compare concentrations of chromatographic peaks between runs, the integrated area over the baseline can be used as a measure.

This area measure is best calculated by online computing integrators, but can also be calculated manually by multiplying the peak height at H/2 bandwidth of the peak.



Example:

In order to compare concentrations of chromatographic peaks between runs where different optical pathlengths have been employed, one must make sure that calibration has been carried out as indicated in the "Calibration".

5. *Factors affecting linearity and quantitive comparisons*

The degree of linearity of a UV-monitor is predominantly a function of how monochromatic the light is. For this reason, monitors which employ light sources yielding a line spectrum, such as from a Hg-lamp, will show superior linearity to monitors employing light sources with continuous spectrum, such as deuterium or xenon lamps.

The non-linear behaviour of UV-monitors is caused by stray light, which can be divided into two portions:

a) stray light directly adjacent in wavelength to monitoring wavelength, due to broadband filters or to the bandwidth of grating plus aperture.

b) stray light not adjacent in wavelength to the monitoring wavelength, due to insufficient blocking capacity of filters or gratings.

Stray light of type a) influences linearity negatively if the sample spectrum shows a sharp incline or decline over the monitoring wavelength, and non-linearity increases in this case with width of stray light adjacent to the monitoring wavelength.

Note that, if the sample spectrum is flat over the monitoring wavelength, this non-linearity does not appear.

Stray light of type b) influences linearity negatively, particularly if the sample is transparent somewhere in the region of the photodetector response. The reason for this is that a small portion (i.e. the stray light) of the light giving 100 % Transmission (i.e. the I_0 in $A = -\log I/I_0$) will always reach the detector regardless of sample concentration, which evidently means that I cannot reach I_0 and thus that a non-linearity shows at high concentrations.

With regard to the stray light problems, LKB employs both at 206 and 254 nm lamps which give spectral emission lines in combination with filters with excellent blocking capacity. Thus the UVICORD S can now show better linearity in the short UV than any other monitor available.

For quantitative comparisons of known sample peaks, the sample spectrum should be studied to ensure that a non-linear behaviour is not likely to occur.

As a guide-line for such a judgement, a comparison can be made with the spectra of uracil and thymine.

The specification of UVICORD S is checked with uracil at 206 and at 254 nm and with thymine at 280 nm.

The response indices are:

at 206 nm, $0.99 < r < 1.01$ between 0 – 1.0A
254 nm, $0.99 < r < 1.01$ between 0 – 1.0A
280 nm, $0.98 < r < 1.02$ between 0 – 0.5A

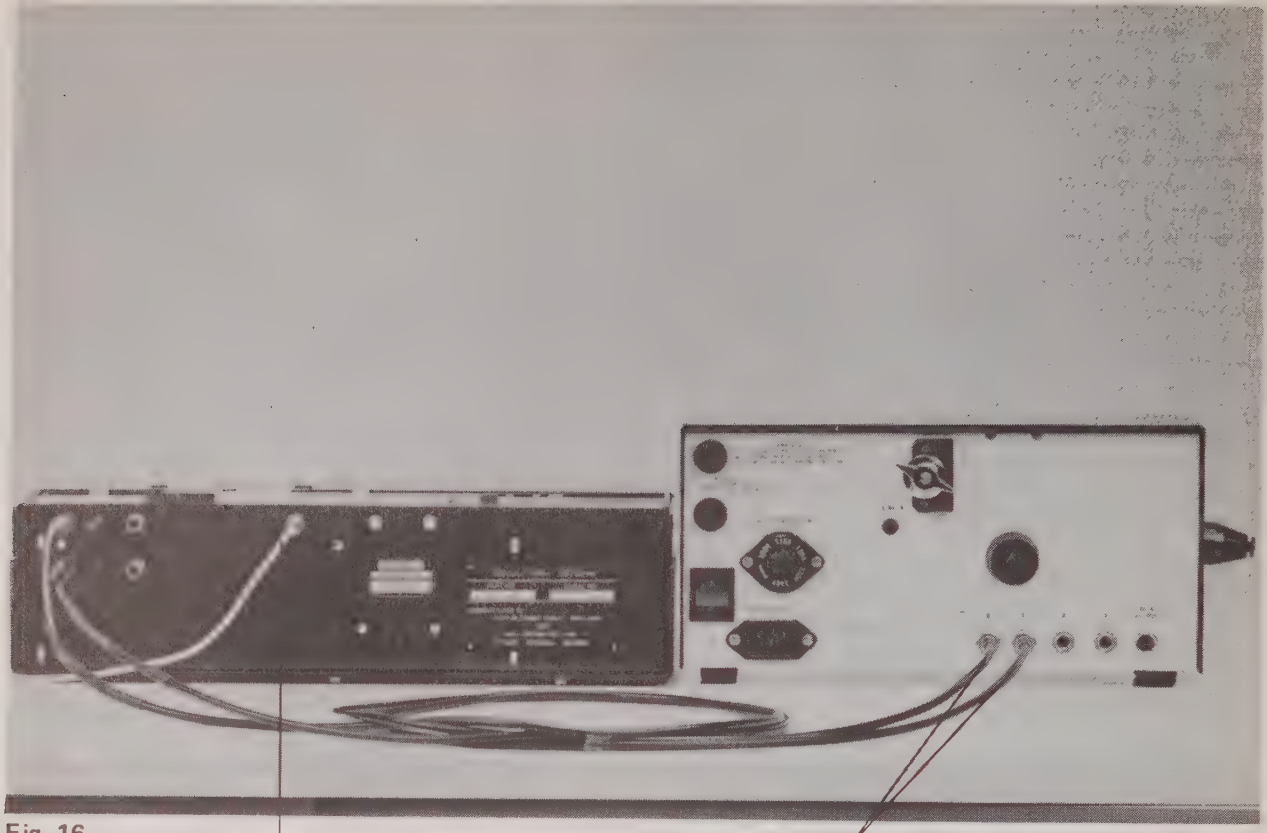


Fig. 16

LKB 2040-105
connecting cable
(for event marking
and pump
control)

LKB 2138-400
recorder cable
(2 cables, both
supplied with
UV-monitor)

LKB 2040-105
connecting cable
(for event marking)

LKB 6523
recorder cable
(3 cables
supplied with
6520-recorder)

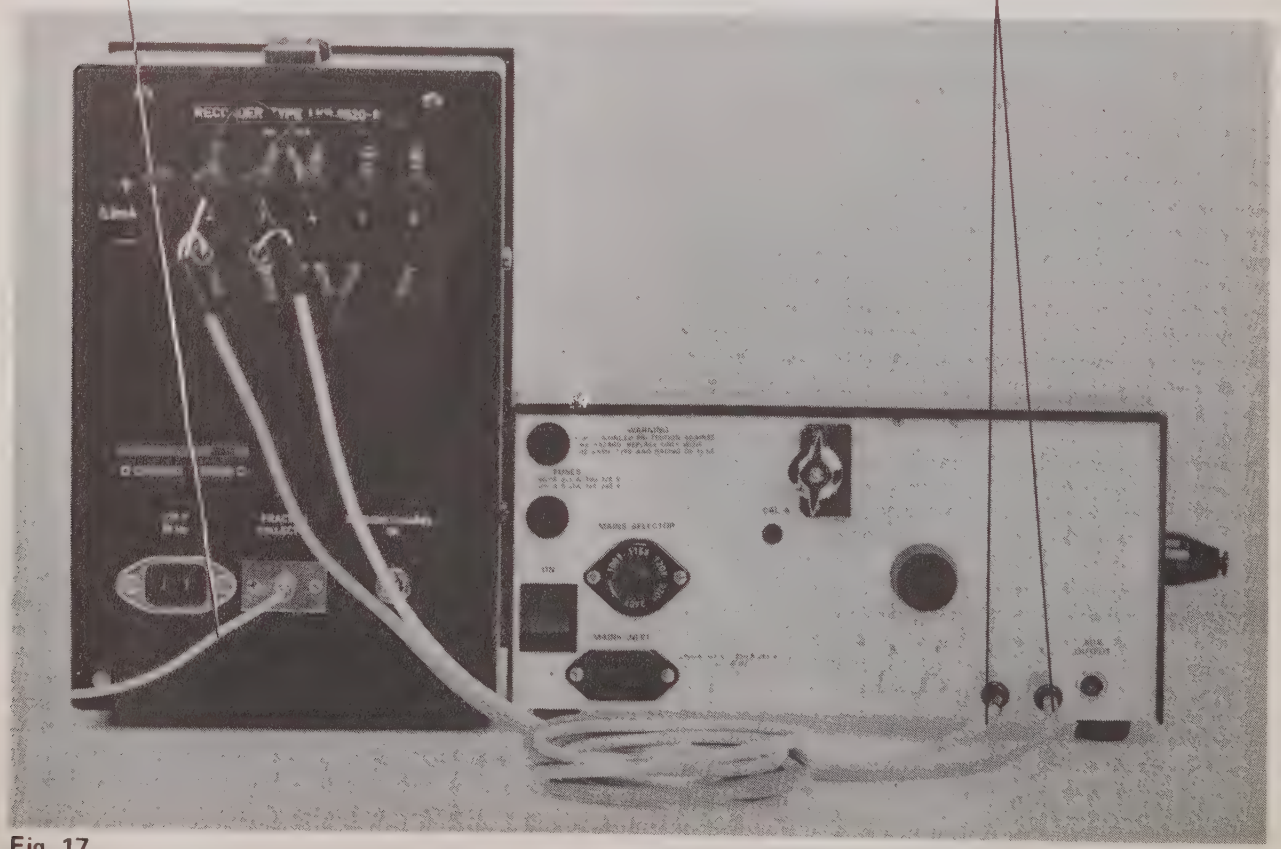


Fig. 17

where r is defined by the relation

$A = K \cdot c^r$

and K is a constant and c concentration.

Should your sample of interest have a 'worse' spectrum, make a calibration curve of absorbance against concentration to ensure correct quantitative readings.

Signal outputs

1. *100 mV-outputs*
   There are two 100 mV-outputs for use with LKB 2210-recorders or equivalents, one output for %T and one for the absorbance range selected with the range selector on the front panel.

   Fig. 16 shows how to connect either or both outputs to a 2-channel 2210-recorder.

   Note: The two recorder cables 2138-400 are supplied with the UV-monitor.

2. *0.3 mA-outputs*
   There are two 0.3mA-outputs for use with LKB 6520-recorders or equivalents, one output for %T and one for the absorbance range selected with the range selector on the front panel.

   Fig. 17 shows how to connect either or both outputs to a 6520 6-channel recorder.

Important note 1: When connecting the 6523 signal cables to the recorder black banana plug must go into red connector on the recorder and red banana plug must go into black connector on the recorder.

Note 2: Three recorder cables 6523A are supplied with the 6520-recorder.

Note 3: In fig. 17 the channel 1 of the recorder is used as event marking channel, connected via an LKB 2040-105 Connecting Cable to either the LKB 2112 RediRac or the LKB 2070 UltroRac® II.

Note 4: If, by mistake, the 100 mV-outputs are connected to a 6520-recorder, the instrument cannot be zero-adjusted, but no damage is caused to either instrument.

Fig. 18

LKB 2040-105
connecting cable
(for event marking
and pump pulse
control)

LKB 2138-400
recorder cable
(2 of these are
supplied with the
UV-monitor)

LKB 2138-410
aux output
recorder cable
(optional
accessory)

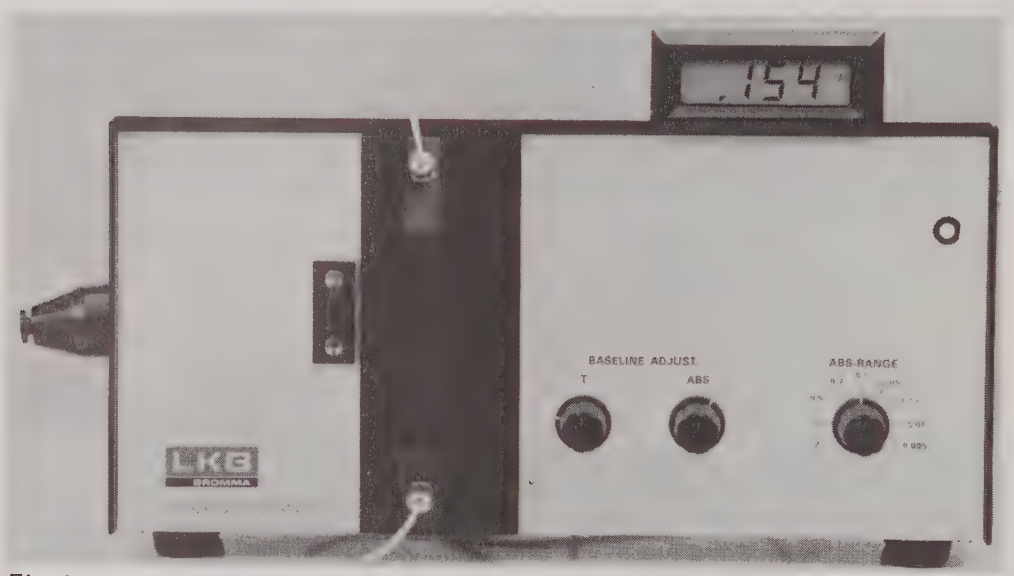


Fig. 19

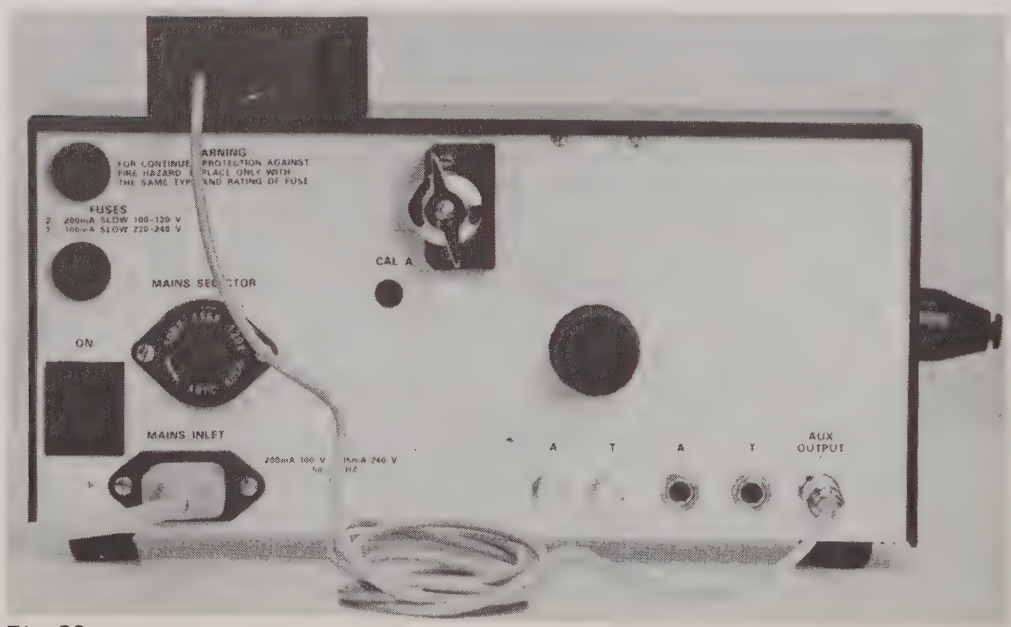


Fig. 20

3. *Auxiliary output as recorder output*
Pin 4 of the auxiliary output gives a – 1 Volt per absorbance unit output, regardless of pre-selected absorbance range. Pin 2 is ground. (For pin connections see Appendix B).

This output can be used to obtain a *second* freely selected absorbance range on the 2-channel 2210-recorder.

When connected to the 1000 mV range on the recorder, the absorbance range equals 0-1.0A. Similarly the:

500 mV-range yields 0–0.5A
200 mV-range yields 0–0.2A
100 mV-range yields 0–0.1A
50 mV-range yields 0–0.05A
20 mV-range yields 0–0.02A
10 mV-range yields 0–0.01A

Naturally any other range between 0.004A and 1.0A can be obtained by using the VAR-function on the recorder.

To connect the AUX. output to a 2210-recorder, an LKB 2138-410 recorder cable must be used (optional accessory).

Note: Since the AUX. output yields –1.00V. per absorbance unit, the recorder must be zeroed to the right and deflection will be to the left.

Fig.18 shows how to connect any two freely selected absorbance ranges to a 2-channel 2210-recorder.

4. *Auxiliary output as integrator output*
The AUX. output yielding –1V. per absorbance unit can be used as signal source for digital integrators.

Connections should be made to:

Pin 4 SIGNAL

Pin 2 GROUND

The appropriate LEMO connector:

LEMO F1 306 Npl 6.5

can be ordered – Order No. 95 86 1000

If the integrator requires a positive signal i.e. +1V per absorbance unit, a small modification can be done to convert the signal. See diagram page 29.

5. *Auxiliary output as digital absorbance read-out output*
If a direct digital absorbance readout is required, connect a digital voltmeter across Pins 4 and 2 as described in 4 above.

As an optional item the LKB 2138-600 LCD Digital Panel Meter can be obtained (see Fig. 19 and 20). This panel meter takes power from the + 15 V DC source available on Pin 1.

APPENDIX A

TECHNICAL SPECIFICATION

($A = -\log I/I_0$ regardless of pathlength).

Operating wavelengths
206 nm, 254 nm and 280 nm (206 and 280 filters supplied as standard)

Absorbance ranges
0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2A.

Linear dynamic range
at 206 nm: 0—1.0A ($0.99 < r < 1.01$)
(measured with uracil)
at 254 nm: 0—1.0A ($0.99 < r < 1.01$)
(measured with uracil)
at 280 nm: 0—0.5A ($0.98 < r < 1.02$)
(measured with thymine)
Note: r is the response index defined by the relation $A = k \cdot c^r$ where k is a constant and c the sample concentration.

Noise level
< 0.0001 A measured peak-to-peak in dry cell

Drift
< 0.0005 A/h at constant temperature

System response time
< 0.5 s for 90 % deflection

Cells
All straight flow-through bubble-avoiding quartz cells. As standard a 2.5 x 5 mm rectangular cell (either pathlength can be used). Cell volume: 70 µl.

Recorder outputs (5)
%T and A for 100 mV-recorders.
%T and A for 0.3 mA-recorders.
— 1 V per 1 A for integrators or voltmeters.

Operating temperature
0 — 30°C

Weight
4.3 kg

Dimensions (LxWxH)
313 x 131 x 147 mm

Power consumption
20 W

Mains voltage requirements
100 — 120 V or 220 — 240 V, 50/60 Hz

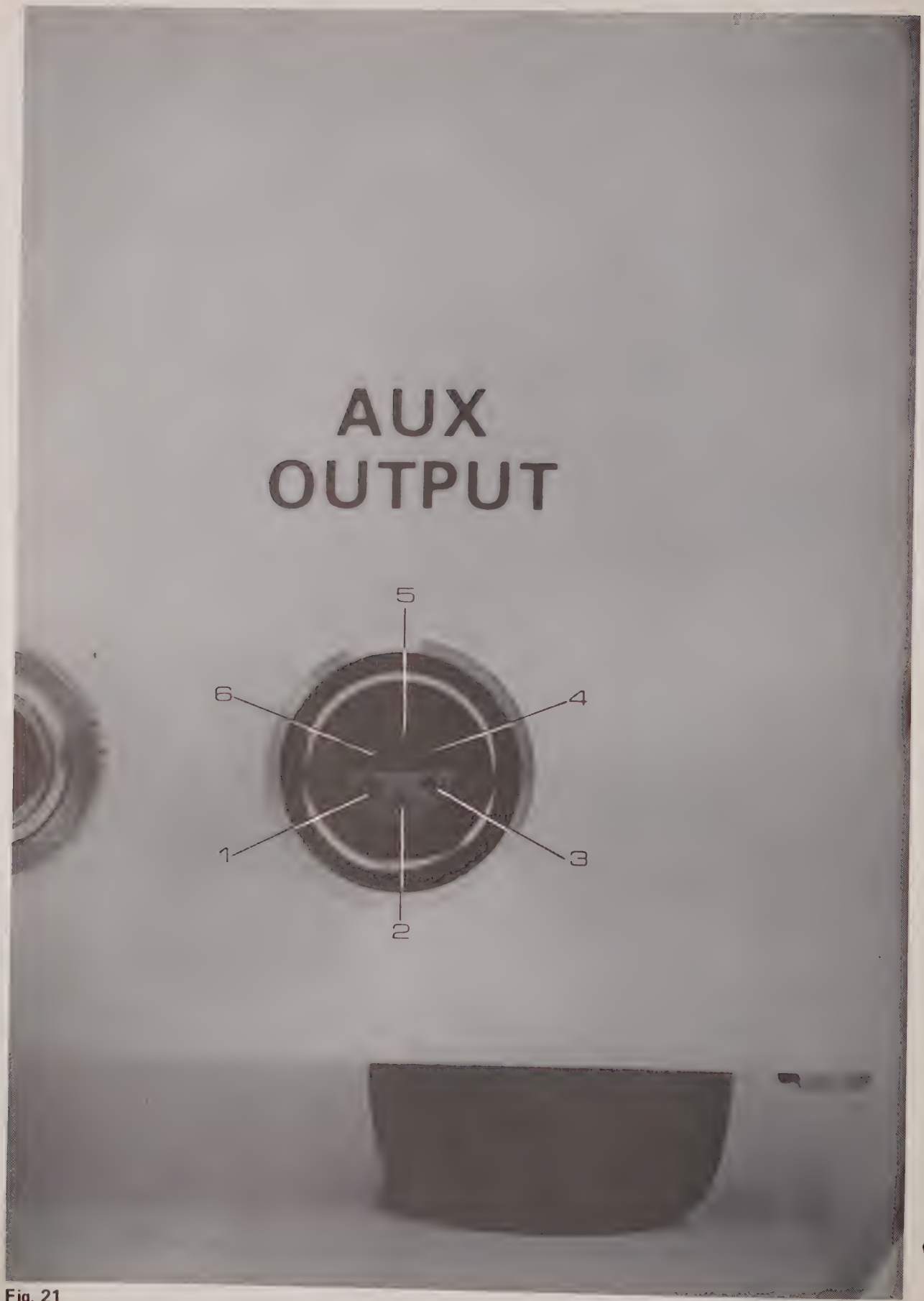


Fig. 21

APPENDIX B

AUXILIARY OUTPUT PIN CONNECTIONS

PIN 1 + 15 V, 10 mA (max)

PIN 2 Ground

PIN 3 − 15 V, 10 mA (max)

PIN 4 Absorbance output voltage level corresponding to absorbance unit i.e. −1.00 V= 1.00 AU independent of range switch position. Source impedance = 10K Ω

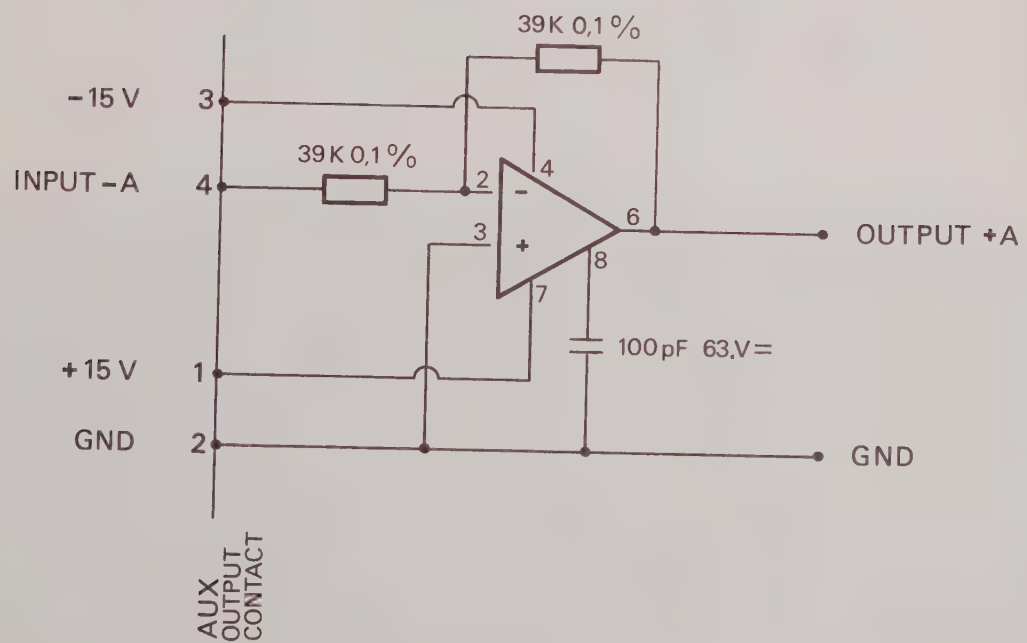
PIN 5 Not connected

PIN 6 Transmission output 0 − 100 mV. Source impedance 25K Ω

The cable contact corresponding to the LEMO connector above has reference No. LEMO F1 306 Npl 6.5 and can be ordered from LKB; spare parts No. 95 86 1000.

2138 UVICORD S
UNITY GAIN INVERTER

OP-AMP LM 308



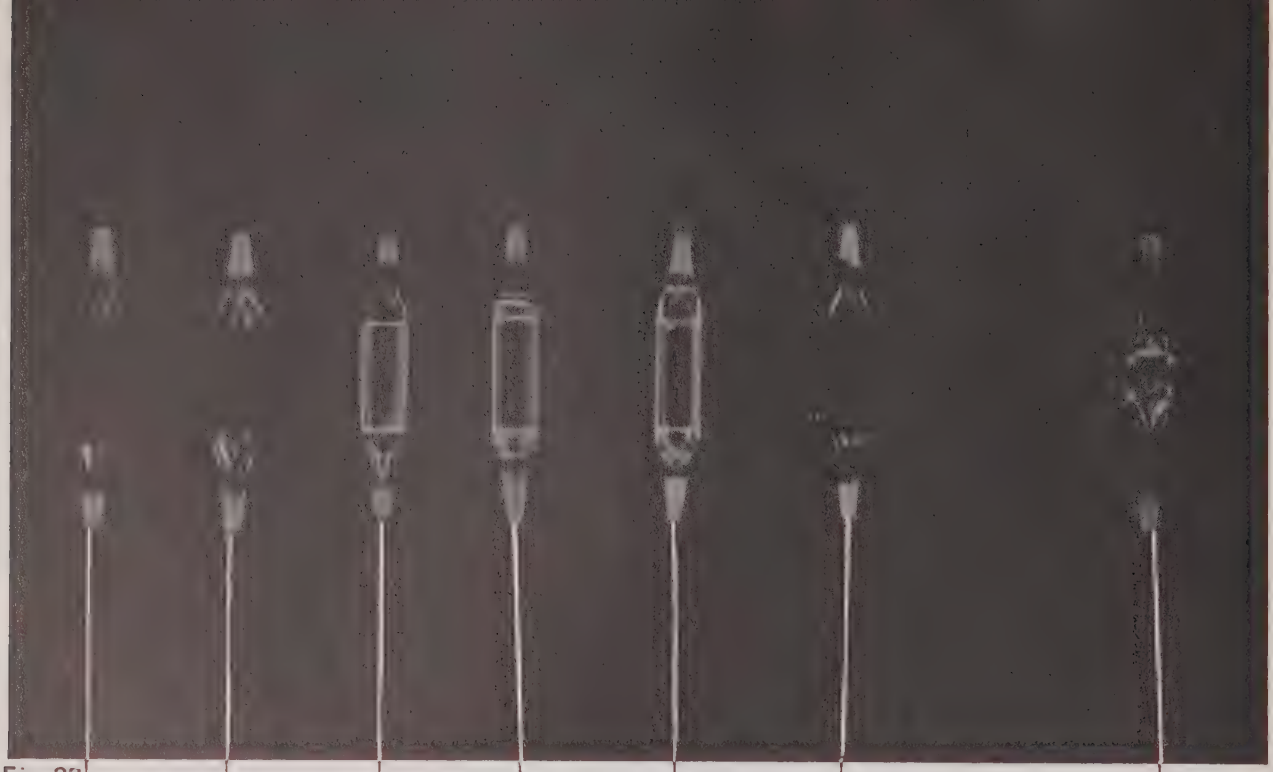


Fig. 22

Note: Optical axis normal to the plane of the page.

4711A-3 4711A-5 4712A-1 4712A-2 4712A-4 4712C-OS 2138-100

Same cells above seen from the side.
Note that the 2138-100 is ground both ways.

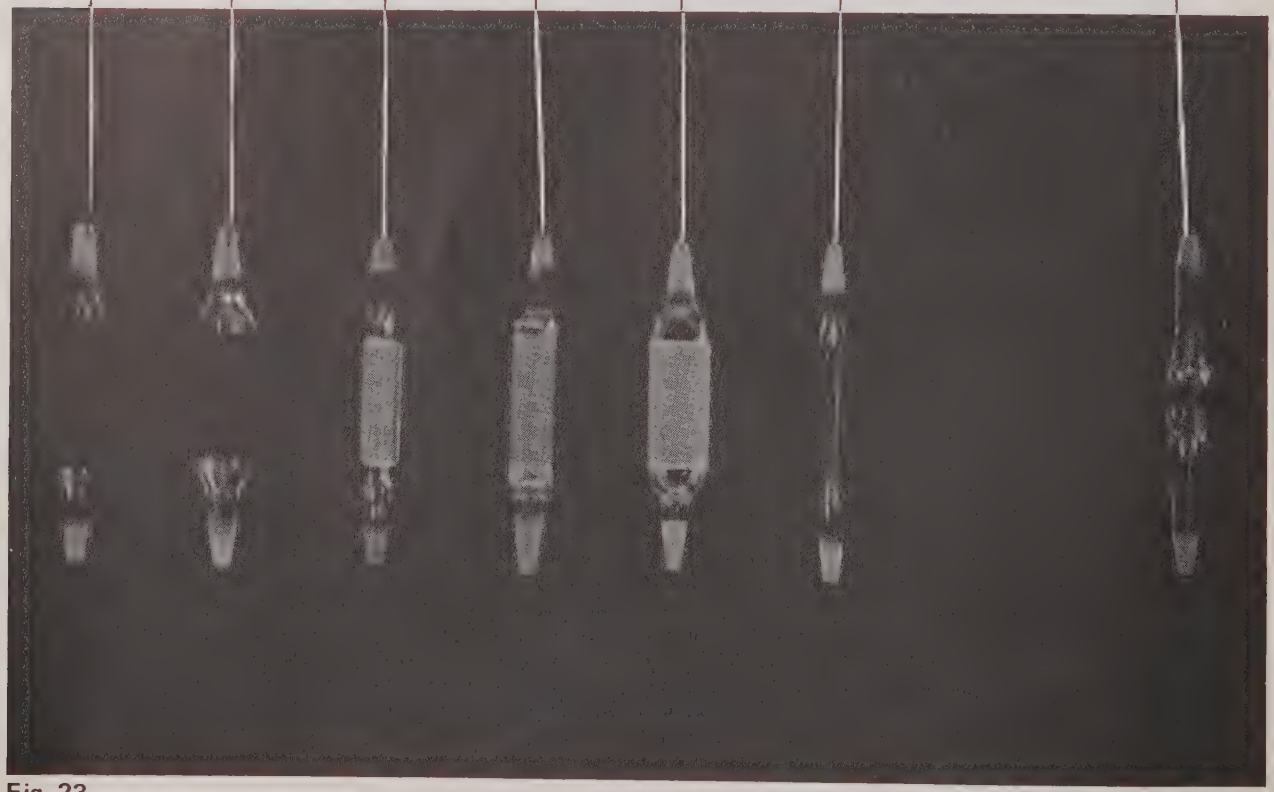


Fig. 23

APPENDIX C.

FLOW CELLS

All LKB flow cells are straight flow-through quartz cells, designed to fulfill requirements regarding inertness, ease of change, ease of inspection, independence of flow rate variations and refractive index effects and having a variety of optical pathlengths.

Available flow cells are:

Flow cells	Opt. pathlength	Volume	Cross section	Max. flow rate at 150 cm water head
4711A-3	3 mm	0.1 ml	circular	1–2 l/h
4711A-5	5 mm	0.3 ml	circular	1–2 l/h
4712A-1	1 mm	0.05 ml	rectangular	1–2 l/h
4712A-2	2 mm	0.1 ml	rectangular	1–2 l/h
4712A-4	4 mm	0.2 ml	rectangular	1–2 l/h
4712C-05	0.5 mm	0.03 ml	rectangular	0.2–0.5 l/h
2138-100	2.5 or 5.0 mm	0.07 ml	rectangular	1–2 l/h

Note: 2138-100 is supplied as standard. This rectangular flow cell can be used in either the 2.5 mm or 5.0 mm optical pathlength directions.

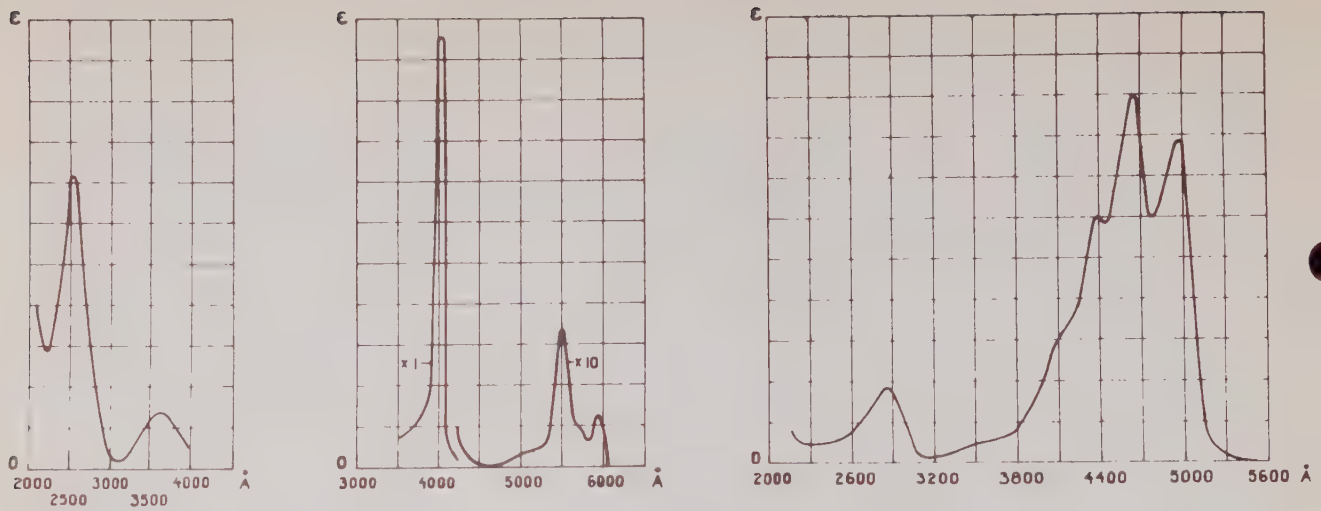


Fig. a

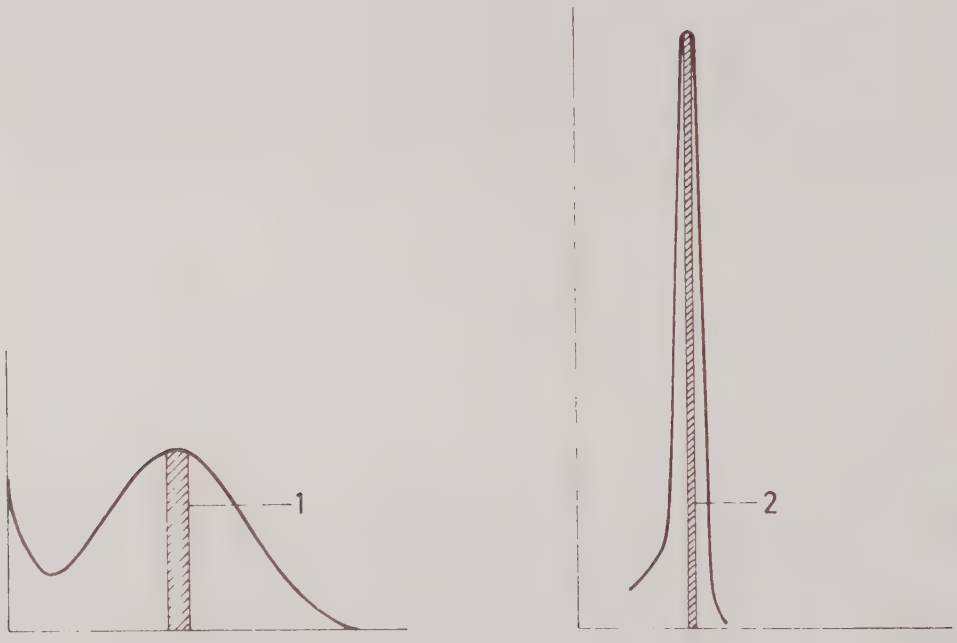


Fig. b

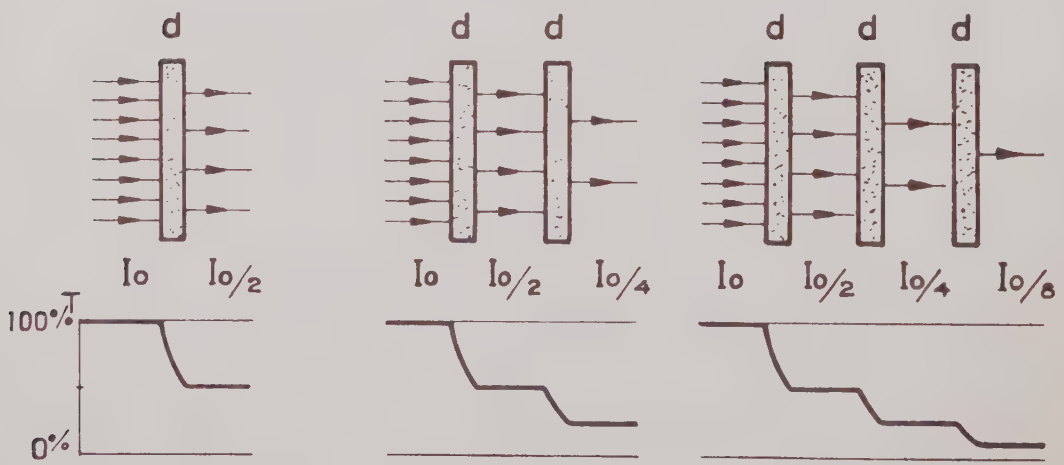


Fig. c

APPENDIX D

Some basic principles in absorption spectrophotometry

Light

Light is an electromagnetic wave motion, the physical, chemical and physiological effects of which depend on the wavelength (λ).

The wavelength is measured in units called Ångströms, named after a Swedish physicist, and abbreviated Å. 1 Å is defined as 1/10,000,000 of 1 mm or 10^{-10} m.
Other frequently used units are millimicron and nanometer, abbreviated mμ and nm. Both these units have exactly the same value. They are 10 times as large as the Ångström unit, i.e. 1 mμ = 1 nm = 10 Å or 10^{-9} m.

The human eye is sensitive to light with wavelengths between approximately 400 and 750 nm. The lower limit corresponds to violet, the upper limit to red light, and in between are distributed the other colours of the spectrum. If all these wavelengths occur together in the relative abundance given by daylight, one speaks of white light. Outside this range of visible light, there are the ultraviolet (λ smaller than 400 nm) and infrared (λ larger than 750 nm).

Spectrum

A spectrum is a picture or curve which shows how the intensity of a light source varies with the wavelengths. A spectrum is said to be continuous if all wavelengths within a certain range exist (sunlight, light from incandescent lamps). A spectrum is said to be *discontinuous* (to be a line spectrum) if only discrete wavelengths exist, with dark fields in between (light from *discharge* lamps). There are also lamps giving superimposed continuous and discontinuous spectra.

Light filters

Filters are made of specially prepared glass (foil contained between glass plates) or cuvettes holding special solutions or gases, which have the ability to absorb (block) some wavelength regions and transmit others. Thus, filters provide a very simple way of isolating a certain colour.
Filtered light from a lamp with a continuous spectrum is a mixture of many wavelengths (polychromatic). Such light usually has a *half bandwidth* of 8 to 25 nm.

On the other hand, a mercury lamp (having a line spectrum) in conjunction with suitable filters can give light of one single wavelength, or *monochromatic* light. This wavelength, however, cannot be freely chosen

Light absorption

When light passes through a medium, a loss of intensity usually occurs. This is called light absorption and it varies considerably with the wavelength, and also of course with the physical nature of the medium. A picture or a curve showing how the light absorption of a substance depends on the wavelength is called its *absorption spectrum*. Examples of such curves are shown in Fig. a, where the left-hand curve refers to formyl-pteroylglutamic acid, the middle one to coproporphyrin, and the right-hand one to γ-carotene.

Measurement of light absorption at different wavelengths is called *absorption spectrophotometry*. As can be seen in Fig. a, absorption spectra are characteristic of the compounds, and recording of such spectra is thus an important aid in identifying chemical compounds.

On the other hand, by measuring the absorption at one single wavelength, one can determine the concentration of a substance provided that no other substances in the sample absorb light at this particular wavelength. It is then better to choose a wavelength which corresponds to a maximum (peak) in the absorption spectrum, partly because there is a maximum in sensitivity at such a point, partly because the absorption is almost independent of the wavelength in a small region at the top of the peak. As a general rule, one can say that the light used for measurement should have a bandwidth appreciably smaller than the width of the absorption band. Thus, the narrower the absorption band, the smaller the required bandwidth, see Fig. b. Of course it is possible to perform measurements on the slope of an absorption peak, but a very small bandwidth is then required.

Definitions in absorption measurements

In absorption measurements it is essential to be familiar with the concepts in current use, which are: The *transmission (transmittance), T*, of a sample at a certain wavelength is defined as that fraction of the incident intensity which is transmitted by the sample. It can also be expressed in per cent.

The *cell thickness, d*, refers to the internal measurement of the cell (the sample thickness) and is thus the distance which the light has to travel in the absorbing medium, it should be given in cm.

The *extinction (absorbance, optical density), E*, is defined as the Briggsian logarithm of the ratio I_0/I between the incident and transmitted light intensities. The extinction is proportional to the concentration of the absorbing substance and to the cell thickness. Thus, at a constant cross-sectional area for the light beam, the extinction is a measure of the mass of absorbing material in the path of the light flux.

The *molar extinction coefficient (molar absorptivity)*, ϵ, is defined as the extinction that would be obtained if the concentration were 1 mole per liter and the cell thickness 1 cm.

The *specific extinction (absorptivity), k*, is defined as the extinction that would be obtained if the concentration is other than 1 mole per liter and the cell thickness 1 cm. The value depends on the concentration unit chosen. Specifically, if the concentration is measured in grams per 100 ml, the specific extinction is often written, not quite logically,* $E^{1\%}_{1\,cm}$. Specific extinctions are used instead of molar extinctions in cases where the molecular weight is unknown or known with an accuracy lower than the one with which the extinction can be measured.

A concentration of 1% strictly means that the solution contains 1 gram of solute for 99 grams of solvent. However, most authors giving concentrations in per cent in connection with extinction measurements probably have in mind the unit 1 gram per 100 ml of solution. Thus it seems to be agreed upon within wide circles that the unit »mg %» actually means 1 mg per 100 ml. Lambert-Beer's law has absolute validity only for concentration units based on volume.

The Lambert-Beer's law

The mathematical relationship between the quantities defined above is expressed by Lambert-Beer's law:

$$I = I_0 - 10^{-kcd} = I_0 \cdot 10^{-E}$$

or in another form:

$$E = kcd = \log I_0/I$$

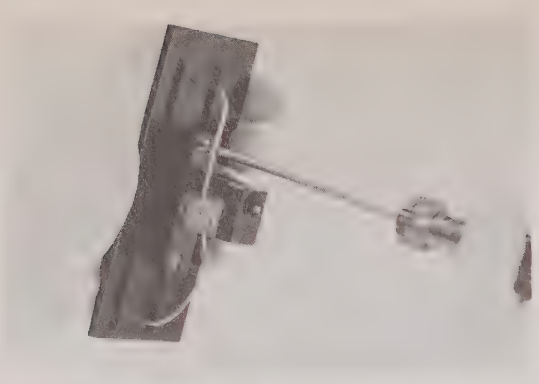
When c is measured in moles per liter, k becomes the molar extinction, E.

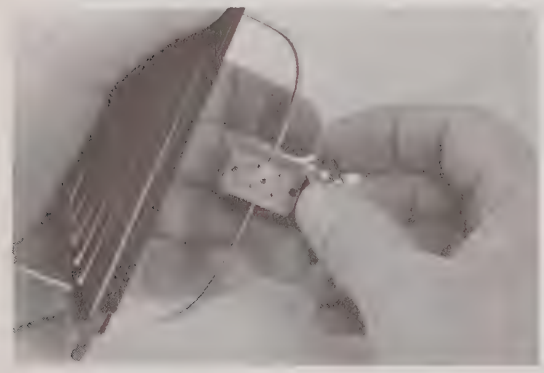
The implications of Lambert-Beer's law are as follows. The relative attenuation which affects the light passing through a medium is independent of the incident light intensity. Consequently, one and the same sample absorbs appreciably more light from a strong source than from a weak one. The phenomenon is illustrated in Fig. c, where three identical absorbing plates have been placed in series. The first plate absorbs 50% of the incident light I_0, the second plate absorbs 50% of what is left, $I_0/2$, and the third absorbs 50% of what has passed the second plate, $I_0/4$. Consequently the extinction varies linearly with the amount of material in the path of the light, while the transmission does not.

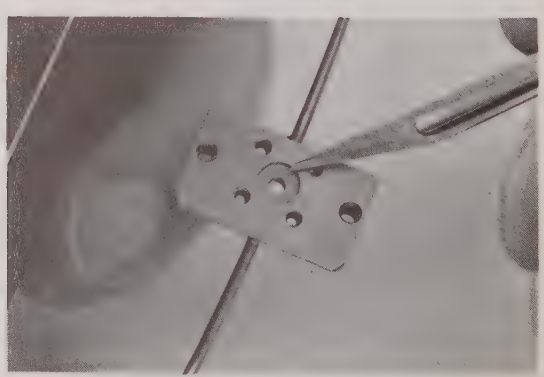
Some practical considerations for evaluation work

1. When the exinction (absorbance, optical density) measured by the UVICORD is recorded, the amount of substance that has flown through the cell can be calculated by measuring the area enclosed between the extinction curve and the baseline, provided that the paper is driven at a speed proportional to the rate of flow. Also the extinction is directly proportional to the cell thickness.
2. When the transmission (transmittance) measured by the UVICORD is recorded, the recorder deflection is not proportional to the concentration, nor to the cell thickness.
   If a double deflection on the recorder is desired, then it is insufficient to double the concentration or the cell thickness (see nomogram on page 22).
   The extinction curve can be constructed from the transmission curve with the aid of the double scale in the nomogram on page 22, or mathematically, by the relationship:

$$E = \log \frac{I}{T} = -\log T$$







APPENDIX E

The HPLC micro-cell

The flow cell holder for HPLC and LC (2138-101 and 2138-102) are interchangeable in the UVICORD S. Thus these paragraphs only relate to what is specific for the HPLC-cell and references are made to the pages of the LC-manual part.

Specifications: All parts are of SS 316, quarts or Teflon®
Cell volume: 8 µl
Tubing (inlet and outlet):
OD 1.59 mm (0,0625")
1D 0.254 mm (0,01")
Pathlength: 2.5 mm

Note I. The cell holder is delivered with two rubber stoppers on the tube ends. Do not remove these until the cell is to be installed in the chromatographic system.

Note II. When setting the cell up it may be necessary to flush the cell thoroughly. Flush at least 50 ml methanol with a rate of 1 ml/min through the cell, preferably with a pump.

I. Detector Plumbing

To avoid detrimental effects from dilution, all connections between injector and the cell inlet should be of the zero dead-volume type.

The HPLC cell is not supplied with any connectors because there are several different types used by different manufacturers. Follow the instructions supplied with the chosen connector thoroughly and be sure not to overtighten the nuts.

The connecting tubes to the HPLC-cell has an ID of only 0,25 mm (0.01"). The bandbroadening in such small tubing is very little compared with the bandbroadening in the HPLC column itself. However to maintain the good resolution achieved in the column it is a good rule to keep even such a small tube as short as possible. Therefore try to connect the column outlet directly to the HPLC cell inlet. The pressure from the column outlet is normally close to the environments, thus it is often appropriate to use Teflon ferrules when connecting the column outlet to the cell.

If for any reason it is necessary to keep a certain pressure in the HPLC cell e.g. to avoid bubble formation in the cell, the best way is to keep the outlet several meters above the cell. Connect a wide teflon tubing to the outlet, (upper tube on the cell) and arrange the waste vessel high. This arrangment will also maintain the pressure in the cell even if the flow is stopped and will also be insensitive to flow fluctuations caused by the HPLC pump.

During installation of UVICORD S or later it may be necessary to cut the stainless steel tubing. This is best done in the same way as one cuts a glass tube. Make a score with a file and bend the tube at the scored point several times in opposite directions until the tube breaks. It is also advisable to take away the burrs around the break with a file but be careful not to file across the opening to prevent filings from falling into the tube.

II. Cleaning of flow cell and tubing

1. *Lack of flow through detector*
If lack of flow from the detector outlet is noted, first confirm that flow is coming out from the column.

If flow is coming out from the column the blockage is in the cell or connecting tubes.

Begin by attaching the pump to the cell *outlet* and try to remove the obstruction by using *reverse* flow through the cell at low pressure. A back pressure of not more than 50 bar (~725 psi) should be put on the cell. If back flushing is not successful remove the quartz window on one side of the cell (see "Cleaning of flow cell") and try again.

If the solvent now flows out through the cell the obstruction must be located in the *inlet* tube to the cell. If the blockage remains it will be located in the *outlet* tube.

Cut either the inlet or outlet tubing (whichever contains the blockage) close to the flow cell allowing sufficient tubing to remain for reconnection. (See "Detector plumbing".) Assemble the cell (see "Cleaning of flow cell") and verify that the obstruction is now removed by feeding solvent from the pump through the cell.

If the blockage still remains the cell assembly with connecting tubes has to be changed. Contact your LKB service representative.

2. *Cleaning of flow cell*
To gain access to the quartz windows first remove the two screws holding the cell assembly in the cell holder (see the pictures on page 36).

Then remove the two screws holding the cell assembly mounting from the handle and take away the cell assembly mounting.

Remove the four screws on one side of the cell assembly and take away the metal plate. Carefully remove the outer small Teflon® washer and shake out the quartz window. Check the inner teflon washer for damage. If damaged, remove with a knife and replace.
Continue in the same way with the opposite window.

If the bore of the assembly contains any visible particles it can be cleaned in an ultrasonic bath with alcohol or simply flushing with a syringe.

Wiping with tissue or swabbing with alcohol is usually sufficient to clean the windows.

Replace the cleaned window in its slot on the top of the bottom washer and place the top washer as before.

Replace the metal plate and lightly tighten the plate on the cell. Quartz is brittle and care should be taken in handling it and in securing the windows. Lightly tighten the screws that are diagonally opposite. Repeat for the other two screws and so on until the four screws are firmly fastend. Pass solvent through the flow cell with a syringe and check for leaks.

Mount the cell assembly in the cell holder and secure with the two larger screws.

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